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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: :EXAMINER: MARVICH

Thomas RITTER, et al. :

SERIAL NO.: 10/068,916 :ART UNIT: 1636

FILED: February 11, 2002 :

FOR: GENETICALLY MODIFIED T-CELLS, METHOD FOR PRODUCING THEM AND USE THEREOF

DECLARATION UNDER 37 C.F.R. §1.132

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

MM 3/5/03

SIR:

Now comes THOMAS RITTER who deposes and states that:

1. I am a graduate of BIOLOGY at ERLANGEN and received my PH.D. degree in the year 1994.

2. I have been employed by CHARITE HOSPITAL for 7,5 years as a SCIENTIST.

3. I am a named inventor of the above-identified application and I am familiar with the prosecution history thereof.

4. The following experiments were performed by me or under my direct supervision and control.

5. The following experiments demonstrate that vIL-10 inhibits the production of the pro-inflammatory cytokine TNF-α by monocytes.

6. To investigate if the secreted cytokine vIL-10 is bioactive after gene transfer, a bioactivity test was established. Due to the high homology between human and viral IL-10

we were able to use a human test system (ELISA, Immulite).

Human peripheral blood lymphocytes (PBMC) were isolated and stimulated with lipopolysaccharide (LPS, 500 pg/ml) which induces the release of the pro-inflammatory cytokine tumour necrosis factor alpha (TNF-α) by monocytes and lymphocytes in the PBMC preparation. The release of TNF-α by monocytes and lymphocytes can be blocked by addition of the anti-inflammatory cytokine human IL-10.

To investigate the inhibitory effects of viral IL-10 on the release of TNF-α, supernatants of vIL-10 transduced cells were added to LPS-stimulated human PBMC in various concentrations and TNF-α production was measured by specific ELISA (Immulite). As shown in the Figure marked as Exhibit 1, addition of vIL-10 containing supernatants inhibited the production of TNF-α approximately 80% at concentrations of 100 ng/ml and 10 ng/ml vIL-10, respectively. At 1 ng/ml of vIL-10, 30% inhibition was observed.

The inhibitory effect of vIL-10 could be blocked by the addition of a neutralizing antibody directed against vIL-10 showing the specificity of the inhibitory effect of vIL-10 on the production of TNF-α.

These results coupled with the data presented in the specification and the common knowledge available demonstrate that one can make the claimed *in vitro* modified T-cells as claimed in this application as well as use those cells in transplantation treatment protocols.

7. While many gene therapy protocols fail to demonstrate sustained expression of a therapeutic gene, two recently published studies using retrovirus-mediated gene transfer demonstrated that transduced cells can be detected after a very long period of time (Cavazzana-Calvo et al. (2000) *Science* 288(5466):669-672; Aiuti et al. (2002) *Science* 296(5477):2410-243--attached as Exhibits 2 and 3).

In experiments conducted either by me or with my supervision, transduced cells (gene

allogeneic

modified T-cells) were also detected over a long-period of time, i.e., greater than 100 days.

In any case, sustained expression of the therapeutic gene might not be necessary in transplantation, because immunomodulation in the early stages of transplantion has been shown to be sufficient in inducing a state of stable tolerance (Qin et al. (1993) *Science* 259(5097):974-977; Lehmann et al. (1997) *Transplantation* 64(8):1181-1187--attached as Exhibits 4 and 5).

While the efficiency of transplantation of transduced allogenic cells is often a problem in many models, the present claims are not directed to allogenic cells or methods of using allogenic cells but *ex vivo* modified autologous cells from the patient. Furthermore, the fact that the cells are autologous contributes to the long life span of these cells *in vivo* because autologous cells do not induce an immune response unlike allogenic cells. Therefore, the efficiency of implantation would not be expected to pose the same problems that are associated with allogenic transplantation and treatment regimens.

While in some instances *in vitro* results may not correlate with *in vivo* results, we have generated *ex vivo* transduced T-cells with essentially the same phenotype as *in vivo* generated regulatory T-cells with respect to over-expressing immunoregulatory molecules, for example, IL-10 secretion, IFN-α secretion, and low expression of CD25, which is described in the present application using, for example, retrovirus mediated gene transfer of therapeutic genes e.g. IL-10, IL-12p40, and others. IL-12p40

Furthermore, it is known that IL-10 has both pro-inflammatory and anti-inflammatory activities but that the EBV-encoded IL-10 (vIL-10) does not possess the stimulatory activities of cellular IL-10 Qin et al. (1997) *Science* 259(509&):974-977; De Waal Malefyt et al., (1991) *J. Exp. Med.* 174(4):915-924), which is also confirmed by the experiments presented in the present application (see Figures 1-3 and the corresponding discussion of those Figures

on pages 21 and 22) and by the data presented in this Declaration, vIL-10 inhibits the production of the pro-inflammatory cytokine TNF-α by monocytes.

I further point out that not all human transplants are established before therapy is begun, which is characteristic of cadaver-donor transplantations. There are numerous transplantations where this is not the case, for example, in living-donor transplantations. For example, kidney liver-donor transplantations constitute approximately 15% of transplantations in Germany, approximately 40% of transplantations in the United States, and greater than 80% in Norway and Japan. Furthermore, even in cadaver-donor transplantations, the responder and donor cells can be collected before starting any immunosuppression and as a result the gene modified T cells can be generated *ex vivo* and used to treat the patients after an initial conventional immunosuppression treatment.

Thus, the phenotype, activities and different types of transplantation as discussed above supports the use of the claimed modified recipient-specific T cells in transplantation treatment regimens.

Regarding the biosafety of retroviral transduced cells, the articles attached as Exhibits 2 and 3 discuss the successful use of retroviral vectors in humans for gene transfer. Furthermore, the present application describes other forms of DNA-transfer such as using with liposomes or by electroporation. Thus, biosafety using the claimed *in vitro* modified T cells are not expected to be problematic.

In view of the above, I conclude that the application as originally filed coupled with the knowledge readily available in the scientific field, demonstrates that one can make the claimed *in vitro* modified T-cells as claimed in this application as well as use those cells in transplantation treatment protocols.

8. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

R RAS

Signature

12.12.02

Date